

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1-116 (Canceled).

117. (New) A library comprising a collection of members of a family; the family comprising a diversity of peptides, polypeptides or proteins; the collection comprising at least a nonbiased portion of the diversity of the family; and the peptides, polypeptides or proteins being encoded by DNA sequences comprising at least in part nucleic acid sequences produced by a method comprising the steps of:

(i) contacting a nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in a region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and

(ii) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the temperature.

118. (New) A library comprising a collection of members of a family; the family

comprising a diversity of peptides, polypeptides or proteins; the collection comprising at least a nonbiased portion of the diversity of the family; and the peptides, polypeptides or proteins being encoded by DNA sequences comprising at least in part nucleic acid sequences produced by a method comprising the steps of:

(i) contacting a nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in a region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a restriction endonuclease recognition site; and

(ii) cleaving the nucleic acid solely at the restriction endonuclease recognition cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;
the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the temperature.

119 (New) A library comprising a collection of members of a family; the family comprising a diversity of peptides, polypeptides or proteins; the collection comprising at least a nonbiased portion of the diversity of the family; the peptides, polypeptides or proteins being encoded by DNA sequences; and the peptides, polypeptides or proteins being produced by a method comprising the steps of:

(i) preparing a collection of nucleic acids that code at least in part for members of the diverse family;

(ii) rendering the nucleic acids single-stranded;

(iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the steps of:

(a) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in a region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and

(b) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide; the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the temperature; and

(iv) expressing a member of the family of peptides, polypeptides or proteins coded, at least in part, by the cleaved nucleic acids and collectively expressing at least a portion of the diversity of the family.

120. (New) A library comprising a collection of members of a family; the family comprising a diversity of peptides, polypeptides or proteins; the collection comprising at least a nonbiased portion of the diversity of the family; the peptides, polypeptides or proteins being encoded by DNA sequences; and the peptides, polypeptides or proteins being produced by a method comprising the steps of:

(i) preparing a collection of nucleic acids that code, at least in part, for members of the diverse family;

(ii) rendering the nucleic acids single-stranded;

(iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the steps of:

(a) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in a region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a restriction endonuclease recognition site; and

(b) cleaving the nucleic acid solely at the restriction endonuclease recognition cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the temperature and at the desired location, and the restriction being carried out using a cleavage endonuclease that is active at the temperature; and

(iv) expressing a member of the family of peptides, polypeptides or proteins coded, at least in part, by the cleaved nucleic acids and collectively expressing at least a portion of the diversity of the family.

121. (New) The library according to any one of claims 117-120, wherein the restriction endonuclease recognition site is for a Type II-S restriction endonuclease.

122. (New) The library according to any one of claims 117-120, wherein the nucleic acid is cDNA.

123. (New) The library according to claim 121, wherein the nucleic acid is cDNA.

124. (New) The library according to any one of claims 117-120, wherein the DNA sequences encode at least a portion of an immunoglobulin.

125. (New) The library according to claim 121, wherein the DNA sequences encode at least a portion of an immunoglobulin.

126. (New) The library according to claim 122, wherein the DNA sequences encode at least a portion of an immunoglobulin.

127. (New) The library according to claim 123, wherein the DNA sequences encode at least a portion of an immunoglobulin.

128. (New) The library according to claim 124, wherein the immunoglobulin comprises a Fab or single chain Fv.

129. (New) The library according to claim 125, wherein the immunoglobulin comprises a Fab or single chain Fv.

130. (New) The library according to claim 126, wherein the immunoglobulin comprises a Fab or single chain Fv.

131. (New) The library according to claim 127, wherein the immunoglobulin comprises a Fab or single chain Fv.

132. (New) The library according to claim 124, wherein the immunoglobulin comprises at least portion of a heavy chain.

133. (New) The library according to claim 125, wherein the immunoglobulin comprises at least portion of a heavy chain.

134. (New) The library according to claim 126, wherein the immunoglobulin comprises at least portion of a heavy chain.

135. (New) The library according to claim 127, wherein the immunoglobulin comprises at least portion of a heavy chain.

136. (New) The library according to claim 132, wherein the heavy chain is an IgM, IgG, IgA, IgE or IgD heavy chain.

137. (New) The library according to claim 133, wherein the heavy chain is an IgM, IgG, IgA, IgE or IgD heavy chain.

138. (New) The library according to claim 134, wherein the heavy chain is an IgM, IgG, IgA, IgE or IgD heavy chain.

139. (New) The library according to claim 135, wherein the heavy chain is an IgM, IgG, IgA, IgE or IgD heavy chain.

140. (New) The library according to claim 132, wherein at least a portion of the heavy chain is human.

141. (New) The library according to claim 133, wherein at least a portion of the heavy chain is human.

142. (New) The library according to claim 134, wherein at least a portion of the heavy chain is human.

143. (New) The library according to claim 135, wherein at least a portion of the

heavy chain is human.

144. (New) The library according to claim 136, wherein at least a portion of the heavy chain is human.

145. (New) The library according to claim 137, wherein at least a portion of the heavy chain is human.

146. (New) The library according to claim 138, wherein at least a portion of the heavy chain is human.

147. (New) The library according to claim 139, wherein at least a portion of the heavy chain is human.

148. (New) The library according to claim 124, wherein the immunoglobulin comprises at least a portion of FR1.

149. (New) The library according to claim 125, wherein the immunoglobulin comprises at least a portion of FR1.

150. (New) The library according to claim 126, wherein the immunoglobulin comprises at least a portion of FR1.

151. (New) The library according to claim 127, wherein the immunoglobulin comprises at least a portion of FR1.

152. (New) The library according to claim 128, wherein the immunoglobulin comprises at least a portion of FR1.

153. (New) The library according to claim 129, wherein the immunoglobulin comprises at least a portion of FR1.

154. (New) The library according to claim 130, wherein the immunoglobulin comprises at least a portion of FR1.

155. (New) The library according to claim 131, wherein the immunoglobulin comprises at least a portion of FR1.

156. (New) The library according to claim 148, wherein at least a portion of FR1 is human.

157. (New) The library according to claim 149, wherein at least a portion of FR1 is human.

158. (New) The library according to claim 150, wherein at least a portion of FR1 is human.

159. (New) The library according to claim 151, wherein at least a portion of FR1 is human.

160. (New) The library according to claim 152, wherein at least a portion of FR1 is human.

161. (New) The library according to claim 153, wherein at least a portion of FR1 is human.

162. (New) The library according to claim 154, wherein at least a portion of FR1 is human.

163. (New) The library according to claim 155, wherein at least a portion of FR1 is human.

164. (New) The library according to claim 124, wherein the immunoglobulin comprises at least a portion of a light chain.

165. (New) The library according to claim 125, wherein the immunoglobulin comprises at least a portion of a light chain.

166. (New) The library according to claim 126, wherein the immunoglobulin comprises at least a portion of a light chain.

167. (New) The library according to claim 127, wherein the immunoglobulin comprises at least a portion of a light chain.

168. (New) The library according to claim 128, wherein the immunoglobulin comprises at least a portion of a light chain.

169. (New) The library according to claim 129, wherein the immunoglobulin comprises at least a portion of a light chain.

170. (New) The library according to claim 130, wherein the immunoglobulin comprises at least a portion of a light chain.

171. (New) The library according to claim 131, wherein the immunoglobulin

comprises at least a portion of a light chain.

172. (New) The library according to claim 164, wherein at least a portion of the light chain is human.

173. (New) The library according to claim 165, wherein at least a portion of the light chain is human.

174. (New) The library according to claim 166, wherein at least a portion of the light chain is human.

175. (New) The library according to claim 167, wherein at least a portion of the light chain is human.

176. (New) The library according to claim 168, wherein at least a portion of the light chain is human.

177. (New) The library according to claim 169, wherein at least a portion of the light chain is human.

178. (New) The library according to claim 170, wherein at least a portion of the light chain is human.

179. (New) The library according to claim 171, wherein at least a portion of the light chain is human.

180. (New) The library according to any one of claims 117-120, wherein the DNA sequences are at least in part derived from patients suffering from at least one autoimmune disease and/or cancer.

181. (New) The library according to claim 180, wherein the autoimmune disease is lupus, erythematosus, systemic sclerosis, rheumatoid arthritis, antiphospholipid syndrome or vasculitis.

182. (New) The library according to claim 180, wherein the DNA sequences are derived at least in part isolated from peripheral blood cells, bone marrow cells, spleen cells or lymph node cells.

183. (New) The library according to any one of claims 117-120, wherein the method further comprises at least one nucleic acid amplification step between one or more of steps (i) and (ii), steps (ii) and (iii) or between steps (iii) and (iv).

184. (New) The library according to claim 183, wherein the amplification step comprises amplification primers that are functionally complementary to a portion of the nucleic acids encoding a constant region.

185. (New) The library according to claim 184, wherein the constant region is a part of the genome of immunoglobulin genes selected from the group consisting of IgM, IgG, IgA, IgE and IgD.

186. (New) The library according to claim 184, wherein the constant region is exogenous to the nucleic acids.

187. (New) The library according to claim 183, wherein the amplification step comprises geneRACEJ.

188. (New) The library according to any one of claims 117-120, wherein the

temperature is between 37°C and 75°C

189. (New) The library according to claim 188, wherein the temperature is between 45°C and 75°C.

190. (New) The library according to claim 189, wherein the temperature is between 50°C and 60°C.

191. (New) The library according to claim 190, wherein the temperature is between 55°C and 60°C.

192. (New) The library according to claim 117 or 119, wherein the length of the single-stranded oligonucleotide is between 17 and 30 bases.

193. (New) The library according to claim 192, wherein the length of the single-stranded oligonucleotide is between 18 and 24 bases.

194. (New) The library according to any one of claims 117-120, wherein the restriction endonuclease is *MaeIII*, *Tsp45I*, *HphI*, *BsaII*, *AluI*, *BlpI*, *DdeI*, *BglII*, *MslII*, *BsiEI*, *EaeI*, *EagI*, *HaeIII*, *Bst4CI*, *HpyCH4III*, *HinfI*, *MlyI*, *PleI*, *MnII*, *HpyCH4V*, *BsmAI*, *BpmI*, *XmnI*, or *SacI*.

195. (New) The library according to claim 194, wherein the restriction endonuclease is *Bst4CI*, *TaaI*, *HpyCH4III*, *BlpI*, *HpyCH4V* or *MslII*.

196. (New) The library according to claim 118 or 120, wherein the length of the single-stranded region of the partially double-stranded oligonucleotide is between 14 and 22 bases.

197. (New) The library according to claim 196, wherein the length of the single-stranded region of the partially double-stranded oligonucleotide is between 14 and 17 bases.

198. (New) The library according to claim 196, wherein the length of the single-stranded region of the partially double-stranded oligonucleotide is between 18 and 20 bases.

199. (New) The library according to claim 118 or 120, wherein the length of the double-stranded region of the partially double-stranded oligonucleotide is between 10 and 14 base pairs formed by a stem and its palindrome.

200. (New) The library according to claim 199, wherein the double-stranded region of the partially double-stranded oligonucleotide comprises a loop of 3 to 8 bases between the stem and the palindrome.

201. (New) The library according to claim 121, wherein the Type II-S restriction endonuclease is *AarI*, *AceIII*, *Bbr71*, *BbvI*, *BbvII*, *Bce83I*, *BceAI*, *BceI*, *BciVI*, *BfiI*, *BinI*, *BscAI*, *BseRI*, *BsmFI*, *BspMI*, *EciI*, *Eco57I*, *FauI*, *FokI*, *GsuI*, *HgaI*, *HphI*, *MboII*, *MlyI*, *MmeI*, *MnII*, *PleI*, *RleAI*, *SfaNI*, *SspD5I*, *Sth132I*, *StsI*, *TaqII*, *Tth111II*, or *UbaPI*.

202. (New) The method according to claim 201, wherein the Type II-S restriction endonuclease is *FokI*.

203. (New) A library comprising a collection of members of a family; the family comprising a diversity of peptides, polypeptides or proteins; the collection comprising at least a nonbiased portion of the diversity of the family; the peptides, polypeptides or

proteins being encoded by DNA sequences; and the peptides, polypeptides or proteins being produced by a method comprising the steps of:

(i) preparing a collection of nucleic acids that code at least in part for members of the diverse family;

(ii) rendering the nucleic acids single-stranded;

(iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the steps of:

(a) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in a region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and

(b) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide; the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the temperature;

(iv) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acids in the region that remains after the cleavage in step (iii) has been effected, and the double-stranded region of the oligonucleotide including any sequence necessary to return the sequences that remain after cleavage into proper and original reading frame for expression and containing a restriction endonuclease recognition site 5' of those sequences that is

different from the restriction site used in step (iii); and

(v) cleaving the nucleic acid solely at the restriction endonuclease recognition cleavage site contained within the double-stranded region of the partially double-stranded oligonucleotide;
the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the temperature and at the desired location, and the restriction being carried out using a cleavage endonuclease that is active at the temperature; and

(vi) expressing a member of the family of peptides, polypeptides or proteins coded, at least in part, by the cleaved nucleic acids and collectively expressing at least a portion of the diversity of the family.

204. (New) The library according to claim 203, wherein the method further comprises at least one nucleic acid amplification step between one or more of steps (i) and (ii), steps (ii) and (iii), steps (iii) and (iv) and steps (iv) and (v).

205. (New) The library according to claim 203 or 204, wherein the DNA sequences encode at least a portion of an immunoglobulin.

206. (New) The library according to claim 205, wherein the double-stranded region of the oligonucleotide encodes at least a part of a framework sequence of an immunoglobulin.

207. (New) The library according to claim 206, wherein the framework sequence comprises framework 1 of an antibody.

208. (New) The library according to claim 207, wherein the framework sequence comprises framework 1 of a variable domain of a light chain.

209. (New) The library according to claim 207, wherein the framework sequence comprises framework 1 of a variable domain of a heavy chain.

210. (New) The library according to claim 206, wherein the framework sequence comprises framework 3 of an antibody.

211. (New) The library according to claim 210, wherein the framework sequence comprises framework 3 of a variable domain of a light chain.

212. (New) The library according to claim 210, wherein the framework sequence comprises framework 3 of a variable domain of a heavy chain.

213. (New) The library according to claim 207, wherein the single-stranded oligonucleotide is complementary to a region outside framework 1.

214. (New) The library according to claim 204, wherein the amplification step comprises amplification primers that are functionally complementary to a portion of the nucleic acids encoding a constant region of the nucleic acids.

215. (New) The library according to claim 214, wherein the constant region is part of the genome of immunoglobulin genes selected from the group consisting of IgM, IgG, IgA, IgE and IgD.

216. (New) The library according to claim 214, wherein the constant region is exogenous to the nucleic acids.

217. (New) The library according to claim 204, wherein the amplification step comprises geneRACEJ.

218. (New) A library comprising a collection of members of a family; the family comprising a diversity of peptides, polypeptides or proteins; the collection comprising at least a nonbiased portion of the diversity of the family; and the peptides, polypeptides or proteins being encoded by DNA sequences comprising nucleic acids that have been cleaved at a desired location by

(i) contacting a nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid at its 5' terminal region; and

(ii) cleaving the nucleic acid solely at the restriction endonuclease cleavage site located in the double-stranded region of the oligonucleotide or amplifying the nucleic acid using a primer at least in part functionally complementary to at least a part of the double-stranded region of the oligonucleotide, the primer also introducing on amplification an endonuclease cleavage site and cleaving the amplified nucleic acid sequence solely at that site;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the temperature.

219. (New) A library comprising a collection of members of a family; the family comprising a diversity of peptides, polypeptides or proteins; the collection comprising at least a nonbiased portion of the diversity of the family; the peptides, polypeptides or

proteins being encoded by DNA sequences; and the peptides, polypeptides or proteins being produced by a method comprising the steps of:

(i) preparing a collection of nucleic acids that code, at least in part, for members of the diverse family;

(ii) rendering the nucleic acids single-stranded;

(iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the steps of:

(a) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid at its 5' terminal region; and

(b) cleaving the nucleic acid solely at a restriction endonuclease cleavage site located in the double-stranded region of the nucleotide; or amplifying the nucleic acid using a primer at least in part functionally complementary to at least a part of the double-stranded region of the oligonucleotide, the primer also introducing on amplification an endonuclease cleavage site and cleaving the amplified nucleic acid sequence solely at that site;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the temperature and at the desired location, and the restriction being carried out using a cleavage endonuclease that is active at the temperature; and

(iv) expressing a member of the family of peptides, polypeptides or proteins coded, at least in part, by the cleaved nucleic acids and collectively expressing at least a portion of the diversity of the family.

220. (New) A library comprising a collection of members of a family; the family

comprising a diversity of peptides, polypeptides or proteins; the collection comprising at least a nonbiased portion of the diversity of the family; the peptides, polypeptides or proteins being encoded by DNA sequences; and the peptides, polypeptides or proteins being produced by a method comprising the steps of:

(i) preparing a collection of nucleic acids that code at least in part for members of the diverse family;

(ii) rendering the nucleic acids single-stranded;

(iii) cleaving the single-stranded nucleic acids at a desired location by a method comprising the steps of:

(a) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in a region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and

(b) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide; the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the temperature;

(iv) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acids in the 5' terminal region that remains after the cleavage in step (iii) has been effected, and the double-stranded region of the oligonucleotide including any sequence necessary to return the

sequences that remain after cleavage into proper and original reading frame for expression; and

(v) cleaving the nucleic acid solely at a restriction endonuclease cleavage site contained within the double-stranded region of the partially double-stranded oligonucleotide, the site being different from that used in step (iii) or amplifying the nucleic acid using a primer at least in part functionally complementary to at least a part of the double-stranded region of the oligonucleotide, the primer introducing on amplification an endonuclease cleavage site and cleaving the amplified nucleic acid sequence solely at that site;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the temperature and at the desired location, and the restriction being carried out using a cleavage endonuclease that is active at the temperature; and

(vi) expressing a member of the family of peptides, polypeptides or proteins coded, at least in part, by the cleaved nucleic acids and collectively expressing at least a portion of the diversity of the family.

221. (New) The library according to claim 219, wherein the length of the single-stranded region of the partially double-stranded oligonucleotide is between 2 and 15 bases.

222. (New) The library according to claim 221, wherein the length of the single-stranded region of the partially double-stranded oligonucleotide is between 7 and 10 bases.

223. (New) The library according to claim 219, wherein the length of the double-stranded region of the partially double-stranded oligonucleotide is between 12 and 100 base pairs.

224. (New) The library according to claim 223, wherein the length of the double-stranded region of the partially double-stranded oligonucleotide is between 20 and 100 base pairs.

225. (New) The library according to any one of claims 218-220, wherein the method further comprises at least one nucleic acid amplification step between one or more of steps (i) and (ii), steps (ii) and (iii), steps (iii) and (iv) and steps (iv) and (v).

226. (New) The library according to any one of claims 218-220, wherein the DNA sequences encode immunoglobulins.